

### REMARKS

In the interest of furthering prosecution Applicants have amended claim 1, to incorporate the features of claim 14.

#### Rejections under 35 USC §103

Claims 1, 10-13, 16-18, 20-21 and 23 remain rejected under 35 U.S.C. 103 as allegedly being unpatentable over Schrecengost et al. (US 6,660,489) in view of Gaylarde et al. (Microbial Contamination of Stored Hydrocarbon Fuels and its Control), further in view of Grubbs et al. (US 4,945,144) or Friswell et al. (US 5,490,872) and Grubbs et al..

Claims 9, 14, 19 and 24 remain rejected under 35 U.S.C. 103 as allegedly being unpatentable over Schrecengost of al. (US 6,660,489) in view of Gaylarde et al. (Microbial Contamination of Stored Hydrocarbon Fuels and its Control), further in view of Grubbs et al. (US 4,945,144) or Friswell et al. (US 5,490,872) and Grubbs et al. as applied to claims 1, 10-13, 16-18, 20-21 and 23 above, and further in view of Calvo Salve of al. (US 5,843,509).

Claims 15 and 22 remain rejected under 35 U.S.C. 103 as allegedly being unpatentable over Schrecengost et al. (US 6,660,489) in view of Gaylarde et al. (Microbial Contamination of Stored Hydrocarbon Fuels and its Control), further in view of Grubbs et al. (US 4,945,144) or Friswell et al. (US 5,490,872) and Grubbs et al. (US 4,945,144) as applied to claims 1, 10-14, 16-18, 20-21 and 23 above, and further in view of Calvo Salve et al. (US 5,843,509), as applied to claims 9, 19, and 24 above, further in view of Green of al. (abstract, Effect of Chemical Sanitizing Agents on ATP Bioluminescence Measurements).

Claims 1,13, 17-18, 20-21, 23-25 stand rejected under 35 USC 103 as being unpatentable over Lundin in view of Schrecengost et al. (US 6,660,489) in view of Gaylarde et al. (Microbial Contamination of Stored Hydrocarbon Fuels and its Control) and Friswell et al. (US 5,490,872).

None of the cited references teach a method which uses lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate as an extractant. As can be seen in table 8 on page 18 of the specification, mixtures comprising benzalkonium chloride (i.e.,

cationic surfactant used in Schrecengost) were tested in comparison to mixtures according the present invention (e.g., containing phosphatidyl inositol and lecithin). The experiments show that the benzalkonium chloride surfactant mixtures were much less suitable for extracting hydrophilic compounds from hydrophobic matixes than the extractants containing phosphatidyl inositol and lecithin.

Schrecengost (US 6,660,489) is silent regarding a capture solution that contains an extractant that is lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate. In addition, Schrecengost teaches the determination of contaminants on a surface or in a food product via an ATP extraction method for biological samples. See for example, the abstract at lines 6 - 8. In column 1, lines 14 - 17, Schrecengost states "the method is particularly useful in the ATP-luciferin-luciferase assay". This assay is commonly used to monitor microorganism contamination in the food manufacturing industry. In column 2, at lines 49-60, Schrecengost points out that it is a "method for extracting ATP from a biological sample" or "for assaying for the presence of ATP in a biological sample" or "for detecting contamination on a surface or in a food product". Food samples are generally composed of organic compounds (e.g., proteins, fatty acids, sugars, salts, organic acids and water). The reference provides no explanation for the meaning of biological sample and no specific food products are discussed. Schrecengost does not teach or suggest detecting an unknown contaminant in a hydrophobic/non-polar/non-ionic liquid matrix (e.g., fuel).

On page 3 of the Office Action, the Examiner concludes "it would have been obvious to one of ordinary skill in the art at the time of the invention to use as the method of Schrecengost with gasoline, aviation kerosene or diesel as the tested product as doing so would have resulted in nothing more than choosing from a finite number of identified and predictable solutions of products at risk of microbial contamination". There is no support for this allegation. A skilled worker would not expect that any microbial test would work with a hydrophobic fluid such as, for example, fuel. There is no way by which the value of such a method can be estimated in advance from its prior use in an entirely different environment (i.e., proteins, fatty acids, sugars, salts, organic acids and water). In the chemical arts even minor changes or variations in the composition of

mediums, even the substitution of an ethyl for a methyl radical, can make a critical difference in the end value or efficacy of a product. As the experiments in Applicant's specification show, even within a hydrophobic matrix different surfactants react very differently.

It is impermissible to use the disclosure as a template to assert obviousness. See, e.g., *In re Fritch* 972 F.2d 1260, 23 USPQ2d 1780 (Fed. Cir. 1992) where the court stated:

It is impermissible to use the claimed invention as an instruction manual or 'template' to piece together the teachings of the prior art so that the claimed invention is rendered obvious." This court has previously stated that "[o]ne cannot use **hindsight** reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention"

Furthermore, throughout the Schrecengost reference it is disclosed that the negatively charged anionic substance acts to neutralize the negative effects of the positive charge of the cationic ATP extractant. See, for example Col. 4 at lines 53-61 which states:

"The present invention provides a method for extracting ATP from a biological sample. In accordance with the extraction method of the present invention, a negatively charged substance, particularly sulfate ion or SDS, is employed during ATP extraction to neutralize the positive charge of commonly used extractants. The anionic substance can be used with extractants such as benzalkonium chloride, benzethonium chloride and dodecyl trimethyl ammonium bromide."

See also Col 2 at lines 64-67; Col. 4 at lines 48-50; Col. 4 at lines 64-67 and Col.5 at lines 10-12.

Thus, Schrecengost does not teach or suggest an extractant that is lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate which improves the yield of the hydrophilic compound extracted from the hydrophobic matrix. Furthermore, it is clear from Schrecengost that the cationic substance is the extractant and the anionic substance acts to neutralize the cationic extractant. A neutralizer implies that something is being added to counterbalance or counteract something else to render it ineffective. Thus, a skilled worker looking towards Schrecengost would not be motivated to use a neutralizing substance in a capture solution. The cationic substance of Schrecengost acts as the extractant. The examiner has not provided any reason why a skilled worker would use a compound taught as a neutralizer, particularly in an amount which improves the yield of the hydrophilic compound extracted from a hydrophobic matrix. There is no teaching to use an anionic substance as an

extractant and a skilled worker would have no expectation that a neutralizer would function effectively as an extractant.

With regards to claim 25, Schrecengost is particularly silent regarding a capture solution that does not contain a cationic extractant. It is only thru the hindsight gleaned from Applicant's specification that a skilled worker would learn that the anionic substance which functions as an extractant improves the yield of the hydrophilic compound extracted from the hydrophobic matrix.

Lundin (US 5,705,345) teaches a method of preparing nucleic acids by obtaining an impure nucleic acid preparation, treating the preparation with a surfactant and adding a cyclodextrin to the treated preparation to neutralize the surfactant. Thus, Lundin is aimed at solving a problem that is entirely different from the problem solved by the present invention. Like Schrecengost, Lundin is not concerned with hydrophobic liquids at all. Lundin is directed towards lysing cells and extracting intracellular components such as DNA, RNA or ATP. In order to avoid interference with substances used for extracting components from cells, Lundin uses cyclodextrin in stoichiometric excess to neutralize the phenol extractant (see, for example, column 6, lines 37- 47). Furthermore, like Schrecengost, Lundin does not teach or suggest an extractant that is lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate which improve the yield of the hydrophilic compound extracted from the hydrophobic matrix.

In contrast, the present invention is directed toward the detection of hydrophilic contaminants, including microbial contaminants, in hydrophobic/non-polar/non-ionic aromatic hydrocarbons ( e.g., kerosene or fuel oil). Moreover, there is no need to destroy microbial cell walls in order to extract intracellular components and there is no need to neutralize an extracting substance in order to avoid interference with the subsequent captured microbial material. Cyclodextrin is not needed for the processing of the claimed method. There is no teaching within Lundin to remove Cyclodextrin from the process and there is no teaching to use an extractant that is lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate. Furthermore, there is no hint within Lundin

that a lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate extractant would improve the yield of the hydrophilic compound extracted from the hydrophobic matrix.

As noted above, neither Lunden nor Schrecengost are concerned with hydrophobic/non-polar/non-ionic aromatic hydrocarbons. To cure this deficiency the Examiner relies upon Gaylarde et al. (Microbial Contamination of Stored Hydrocarbon Fuels and its Control) which discusses the problem of microbial contamination of fuel supplies. Gaylarde, simply teaches a skilled worker that stored fuels can be contaminated with microbial contamination. Gaylarde is completely silent on how to test for such contamination. It would not have made obvious to use the method of Schrecengost or Lunden with gasoline, aviation fuel, kerosene or diesel, because the mixtures disclosed by Schrecengost and Lunden are not suitable for extracting hydrophilic compounds from hydrophobic matrices.

As can be seen in table 8 on page 18 of the specification, mixtures comprising benzalkonium chloride (i.e., cationic surfactant used in Schrecengost) were tested in comparison to mixtures according to the present invention. The experiment shows that the benzalkonium chloride surfactant mixtures were much less suitable for extracting hydrophilic compounds from hydrophobic matrices than the extractants of the present invention. See, page 18 at lines 29-31.

Furthermore, even with the knowledge derived from Gaylarde et al (i.e., that fuel supplies can be contaminated by microbes) a skilled worker would not be led to select the test disclosed by Schrecengost, which tests food contamination.

The Examiner relies upon Grubbs (US 4,945,149) for its teaching regarding a cationic extractant, methylene blue. However, like Schrecengost and Lunden, Grubbs has nothing to do with hydrophobic/non-polar/non-ionic liquid matrixes. Also, Grubbs does not teach methylene blue as a cationic extractant as alleged. Grubbs discloses a chemical reaction (i.e., polymerization of 7-oxan-orborene and its derivatives). Water is used as a solvent and methylene blue is used for complex building with polymers. In contrast to Grubbs, in the present invention dyes are added for an entirely different purpose, to visualize the aqueous phase and not for complex building with polymers. See, for example Col. 10 at lines 45 - 51 of Grubbs which discloses that the polymers

prepared show flexible binding cavities which are suitable to coordinate with Na<sup>+</sup>, K<sup>+</sup>, and Cs<sup>+</sup> salts and preferentially complex large polyaromatic cationic dyes such as methylene blue and rhodamine 6G.

Thus, even if the disclosure of Grubbs is combined with that of Schrecengost the skilled worker would not glean any information regarding the problem solved by the present invention (i.e., how to separate hydrophilic compounds like biological material or particles from a hydrophobic/non-Polar/non-ionic liquid).

Friswell (US 5,490,872) discloses suitable markers for the tagging of petroleum, which are only visible if they are extracted in acidic aqueous solution. These markers are useful for the distinguishing of commercial aromatic liquids. However, Friswell does not cure the deficiencies of the primary references .

Green et al. (Abstract, Effect of Chemical Sanitizing Agents on ATP Bioluminescence Measurements) does not add to the teachings of the primary references. Green et al. is relied upon for its teaching regarding sodium hypochlorite as a sanitizing agent that would not negatively affect ATP bioluminescence measurements.

Thus, Friswell (US 5,490,872) and Green et al. do not cure the deficiencies of Gaylarde and Schrecengost in view of Grubbs. None of the references teach or suggest a method for detecting a hydrophilic compound or biological material dispersed in a hydrophobic/non-polar/non-ionic liquid matrix.

Schrecengost, Lunden, Friswell, Gaylarde, Grubbs and Green are silent regarding a capture solution that contains an extractant that is a lecithin, phosphatidyl inositol, deoxycholic acid, or potassium sorbate. To cure this deficiency the Examiner relies upon Calvo Salve (US 5,843,509) which teaches that lecithin is a natural surfactant. However, Schrecengost, Lunden and Grubbs already teach extractants which work for their particular applications. No deficiencies in the prior art extractants were noted. The Examiner has not provided any reason why a skilled worker would be motivated to make the substitution to a natural surfactant such as lecithin. The prior art does not provide a skilled worker with any hint that the extractants of the present invention improve the yield of a hydrophilic compound extracted from a hydrophobic matrix.

Furthermore, Calvo Salve discloses the use of positively charged water-soluble polysaccharides and a negatively charged phospholipid in a stabilized colloidal system. There no relationship whatsoever between the subject matter of the present invention and the teachings of Calvo Salve, which relate to coatings which are the product of phospholipids (such as lecithin) and an aminopolysaccharide.

In the present invention, there is no need for creating stabilized colloidal systems. The disclosure of Calvo Salve is in an entirely different field and a skilled worker would not logically look towards the Clavo Salve reference for guidance on a method to detect hydrophilic compounds in fuel.

It is only thru the hindsight gleaned from Applicant's specification that a skilled worker would learn that, for example, lecithin or phosphotidyl inositol functions as an extractant which improves the yield of the hydrophilic compound extracted from the hydrophobic matrix.

On page 11 of the Office Action the Examiner refers to Applicants previous comments regarding to the comparative Examples in the specification and states "this does not address the combination of Gaylorde and Schrencongost as the references do not teach this". As noted above, Schrecongost teaches extractants such as benzalkonium chloride, benzethonium chloride and dodecyl trimethy ammonium bromide. At table 8 on page 18 of the present application benzalkonium chloride, a cationic surfactant, is added to the capture mixture. Tables 7 and 8 summarize the results of experiments with surfactants that are different from the extractants of the present invention. The comparative examples in tables 7 and 8 do not lead to recovery ratios as high as the examples according to the present invention which are shown in tables 5 or 6. The recovery percentages in table 8 are much lower.

Thus, based on the above remarks reconsideration of the rejections is respectfully requested.

The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,  
/Anthony Zelano/

Anthony J. Zelano, Reg. No. 27,969  
Attorney for Applicants

/Jennifer Branigan/

Jennifer J. Branigan, Reg. No. 40,921  
Agent for Applicants

MILLEN, WHITE, ZELANO & BRANIGAN, P.C.  
Arlington Courthouse Plaza 1  
2200 Clarendon Boulevard, Suite 1400  
Arlington, VA 22201  
Telephone: 703-243-6333  
Facsimile: 703-243-6410  
Attorney Docket No.: MERCK-3133

Date: 12 April 2011  
JJB:K:\Merck\3000 - 3999\3133\reply April 2011.doc